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From Sado Valley to Europe: Mesolithic dietary practices through different geographic distributions

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ABSTRACT

This study presents new stable isotope (carbon and nitrogen) data from human and faunal remains from three Mesolithic shell middens (Cabeço das Amoreiras, Arapouco and Cabeço do Pez), located on the estuary of the Sado River, Portugal. The results have revealed a diet composed mainly of terrestrial C3 resources (from terrestrial animals and a small contribution from vegetable sources) and a proportion of marine resources close to 20%. These groups followed a subsistence pattern characterized by a variable settlement regime promoted by the availability of the resources in each region, and social and demographic factors that would induce human dietary diversification.

The Sado Valley results were compared with other European Mesolithic groups in order to provide a general view of the subsistence patterns of some of the last hunter-gatherer groups. The high degree of regionalization observed with the comparisons shows that it is impossible to characterise a single subsistence pattern for all European Mesolithic groups. In this sense, environmental characteristics, the geomorphology, the effectiveness of communities' adaptation, and the influence of social and demographic factors probably influenced Mesolithic subsistence patterns in Europe.

1. Introduction

The Mesolithic period is of particular interest in archaeology because it represents the last communities whose subsistence was based exclusively on hunting, fishing and gathering. This period saw the end of the general hunter–gatherer economic pattern in Europe, although there were some communities that followed this subsistence economy throughout the Neolithic period (Bollongino et al., 2013). In this regard, the extensive study of European Mesolithic communities provides information not only about a lifestyle that almost disappeared millennia ago, but also about the processes that took place during the change in the subsistence economy that led to the appearance of the first farming and herding societies.

Therefore, the study of European Mesolithic human remains provides details of individual subsistence patterns, which enables the inference of their adaptations to the environment and some of the social factors that define this period. Unfortunately, in many parts of Europe (especially in warmer regions such as Southern Europe), human remains from the Mesolithic are scarce and the collagen may be poorly preserved. Nevertheless, in some areas, such as Portugal, a great number of human remains have been discovered. Portuguese Mesolithic communities have generally been studied in relation to the shell middens at Muge, where more than 300 individuals have been recovered (Lubell et al., 1994).

Understudied and less well known, the complex of Sado Valley shell middens comprises eleven sites, of which six (Cabeço das Amoreiras, Arapouco, Cabeço do Pez, Poças de São Bento, Vale de Romeiras and Várzea da Mó) have yielded a total of 112 human skeletal remains (Cunha and Umbelino, 2001; Cunha, 2002–2003; Umbelino, 2006). The archaeological excavations of Sado shell middens were performed in the early and mid-twentieth century

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Fig. 1. Location of the Mesolithic sites in Sado Valley presented in this study.

and, regrettably, there is no publication of the fieldwork except for the excavation plan drawings of Cabeço das Amoreiras made by Mr. Dario de Sousa (Arnaud, 1989; Umbelino and Cunha, 2012). Recently, new projects are being carried in both Muge and Sado shell middens, and these will surely provide valuable new data in the next few years.

In this context, the main goal of the present study is to obtain new data on the subsistence pattern of some of these less-studied Mesolithic communities in Western Iberia, the Sado Valley complex. The dietary evidence of Sado Valley groups obtained from carbon and nitrogen stable isotope analysis allows their subsistence pattern to be established and that will lead to a better understanding of regional settlement and the use of estuarine landscapes in the Mesolithic. Subsequently, with the aim of characterizing European Mesolithic communities' subsistence, the data obtained in this study will be compared with data from other contemporary sites in the Iberian Peninsula and at other European Mesolithic sites. These comparisons will be carried out taking into account the environmental conditions in each geographic region.

2. The study sites: location, funerary structures and burial practices

The Sado Valley sites are located on the estuary of the Sado River (Fig. 1), approximately 40 km south of Lisbon in the province of Alentejo (Portugal). The studied sites, Cabeço das Amoreiras, Arapouco and Cabeço do Pez are located near the Sado shore, at the edges of a Miocene plateau, at an altitude of 40–50 m above sea

level (Arnaud, 1987; Araújo, 1999). Várzea da Mó, a fourth site under study, is located at altitudes of 15–20 m, on the left shore of Alagalé River, a small tributary of the Sado River, about 2 km from its confluence (Araújo, 1999). The burials were found in shallow shell middens with a depth of between 0.2 and 0.7 m, since they were mainly spread over an area, and a somewhat irregular shape (Arnaud, 1986).

According to Arnaud (1989) and Cunha and Umbelino (1995–1997, 2001), little information can be obtained from fieldwork related to the burial practices, but there does not seem to be a common pattern either for the main orientation of the bodies (random, east-west, west-east), nor for their position (foetal, lateral position, etc.). Most burials were single, with the exception of two double burials, always with a woman and a child (Cunha and Umbelino, 1995–1997, 2001).

3. Carbon and nitrogen isotope analyses for palaeodietary reconstructions

Stable carbon and nitrogen isotope analysis is a highly useful tool that provides information about the importance of each type of protein intake in the diet of the individuals. The analysis of these two isotopes is commonly used to reconstruct past human and faunal diets and to determine the subsistence strategies of the communities.

In palaeodietary research, the stable carbon isotope value ($\delta^{13}C$) indicates the frequency of marine vs. terrestrial protein input into the diet, with endpoints of ca. −21‰ to −20‰ for a purely terrestrial protein diet and ca. −12‰ for pure marine protein consumption. This value is also useful to distinguish between different dietary components such as C_3 and C_4 plants and the animals that consume them (Schoeninger and De Niro, 1984; Schwarcz and Schoeninger, 1991; Richards, 2002; Lillie, 2003; Eriksson et al., 2008; Lillie et al., 2011). Since edible C_4 plants were presumably not present in Western Europe during the Mesolithic (Sage et al., 1999), the main use of carbon isotopes is to estimate the consumption of marine vs. terrestrial protein in the individual's diet. Mixtures of marine and freshwater food may yield $\delta^{13}C$ values that simulate terrestrial food, but this ambiguity can, to some extent, be resolved through measurement of stable nitrogen isotope values ($\delta^{15}N$). The $\delta^{15}N$ value provides information on the organism's trophic level: the values of consumers present an increase of ca. 3–5‰ over the values of their diets (De Niro and Epstein, 1981; Schoeninger and De Niro, 1984). This increase up the food chain makes nitrogen stable isotopes useful for detecting the presence of high trophic level marine and freshwater foods in the diet (Schoeninger et al., 1983) as well as for distinguishing diets with an important animal component from plant-rich diets (Minagawa and Wada, 1984), but always taking into account the limitations mentioned in some recent studies such as Hedges and Reynard(2007) and Warinner et al. (2013).

Bone collagen is commonly used for carbon and nitrogen stable isotope analysis because it is the predominant protein in the skeletal tissue and has a slow turnover. Since bone is constantly being remodelled during the individual's lifetime, the stable isotope signature of an adult human bone reflects the average diet representative of the last 10–15 years prior to death (Hedges et al., 2007). Established quality indicators are used to ensure the preservation quality of the extracted collagen (De Niro, 1985; Ambrose, 1993; Van Klinken, 1999). $\delta^{15}N$ values from bone collagen reflect only dietary proteins but not the whole dietary spectrum (Ambrose and Norr, 1993) since $\delta^{13}C$ values may be derived from other dietary macronutrients like lipids and carbohydrates (Howland et al., 2003; Jim et al., 2006).

It is important to note that both carbon and nitrogen isotope values from consumers' bone collagen are higher than the corresponding values of their prey (Fischer et al., 2007), and additionally the climate effect can vary the $\delta^{13}C$ value of plants and, consequently, could vary the $\delta^{13}C$ values of all the trophic levels that come after them (Van Klinken et al., 1994; Hedges et al., 2004). Therefore, it is preferred that the terrestrial and marine endpoints are determined in the same location as the contemporary fauna. In this regard, the generally agreed-upon offsets of approximately 1‰ for $\delta^{13}C$ and 3–5‰ for $\delta^{15}N$ have been adopted (e.g. Masao and Wada, 1984; Schoeninger and De Niro, 1984; Richards and Hedges, 1999; Bocherens and Drucker, 2003; Fischer et al., 2007).

4. Material and methods

The stable carbon and nitrogen isotope analysis was performed using bone collagen extracted from 29 samples of skeletal remains: 18 human individuals and 11 faunal bone samples. Bone samples from 4 humans and 10 animals from Cabeço das Amoreiras, 4 humans and 1 animal from Arapouco, 9 humans from Cabeço do Pez and 1 human from Várzea da Mó, were sampled. All individuals were adults; except for 2 children (1.5–2 and 5–7 years of age) from Cabeço do Pez. These sites date from 6400 to 5450 cal BC, according to the radiocarbon dating of human remains (Table 1).

The samples were taken at the National Museum of Archaeology in Lisbon, where the human remains had been deposited and preserved in a paraffin block after the archaeological excavations during the 1920s. No preservation methods were used in the field to preserve faunal remains. The sampling process was conducted at the museum, carefully selecting bone fragments without paraffin and prioritizing fragments of long bones from the lower extremities. All human and faunal individuals available were sampled.

Even though some bones were covered with paraffin, the anthropological study of the skeletal remains, and thus the

Table 1
Radiocarbon dates from the four Sado Valley sites analysed in this study.

Site	Sample	^{14}C age BP	^{14}C age BC (Calibrated 2σ)	Source
Cabeço das Amoreiras	*Skeleton 5* (Beta-125110)	7230 ± 40	6220–6010	Cunha and Umbelino (2001)
	Shells (Q-AM85B2b)	5990 ± 80[a]	5200–4600	Araújo (2003)
	Charcoal (Q-AM85B2a)	5990 ± 75	5070–4710	Araújo (2003)
Arapouco	*Skeleton 2A* (Sac-1560)	7200 ± 130	6400–5800	Cunha and Umbelino (2001)
	Shells (middle layers Q-2492)	7040 ± 70[a]	6450–5700	Araújo (2003)
Cabeço do Pez	*Skeleton 4* (Sac-1558)	6740 ± 110	5850–5470	Cunha and Umbelino (2001)
	Skeleton 4 (Beta-125109)	6760 ± 40	5730–5610	Cunha and Umbelino (2001)
	Shells (middle layers Q-2497)	6450 ± 80[a]	5530–5000	Araújo (2003)
	Shells (middle layers Q-2496)	6150 ± 70[a]	5260–4690	Araújo (2003)
Várzea da Mó	Shells (middle layers ICEN-273)	7110 ± 50	6170–5460	Arnaud (2000)

[a] These dates were corrected for the reservoir effect through the subtraction of 380 ± 30 years (Soares, 1995).

diagnosis of sex and age, could be carried out. Sex diagnosis was made through the study of the pelvic bone (Buikstra and Ubelaker, 1994; Ferembach et al., 1980). Estimation of age at death for adults was based, when possible, on the auricular surface of the ilium (Lovejoy et al., 1985), the closure of cranial sutures (Masset, 1982) and tooth wear (Brothwell, 1989). For non-adults, the method was based on the sequence of formation and eruption of teeth (Ubelaker, 1989), on the union of epiphyses and diaphyses (Ferembach et al., 1980) and on the length of long bones (Scheuer and Black, 2000).

The chemical analyses of the Sado Valley material were carried out in the laboratories of the Biological Anthropology Department at the Universitat Autònoma de Barcelona. Prior to analysis, mechanical abrasion with a Dremel Diamond Engraving tip was used to remove visible contaminants. Collagen extraction proceeded following Richards and Hedges (1999), including Brown et al.'s (1988) modifications referring to the ultrafiltration step.

The measurement of the stable carbon and nitrogen isotope ratios were performed in duplicate in the laboratories of the Institute of Environmental Science and Technology at the Universitat Autònoma de Barcelona, using a Thermo Flash 1112 elemental analyser coupled to a Thermo Delta V Advantage mass spectrometer with a Conflo III interface. This measures the ratios of $^{13}C-^{12}C$ and $^{15}N-^{14}N$ relative to a standard (V-PDB for carbon and AIR for nitrogen), and expresses the stable isotope values in delta notation (δ) in parts per thousand (‰). The international laboratory standard, IAEA 600 (caffeine), is used here. An analytical error always below 0.2‰ (1σ) in all the repeated analyses was determined for $\delta^{13}C$ and $\delta^{15}N$. The statistical analyses were performed using the IBM Statistics Software SPSS 21.0. Tests were performed separately for adults and sub-adults.

5. Results

The isotope ratios obtained from human and faunal skeletal material from the Sado sites are presented in Tables 2 and 3, and are graphically represented in Fig. 2. 11 out of a total of 18 human remains sampled and 2 out of 11 faunal remains sampled yielded enough collagen for analysis in duplicate and passed the collagen quality control criteria (De Niro, 1985; Ambrose, 1993; Van Klinken, 1999) indicating good preservation. With a single human sample, the site of Várzea da Mó is completely excluded from all the summary calculations and figures, as well as 1 human sample and 9 faunal samples from Cabeço das Amoreiras, 3 human samples from Arapouco, and 2 human samples from Cabeço do Pez.

The only 2 faunal values obtained from Sado Valley were *Bos primigenius* Sample 3 from Cabeço das Amoreiras and *Sus* sp. from Arapouco. The $\delta^{13}C$ values of the herbivore (−19.0‰) and the omnivore (−21.8‰) are typical values for a terrestrial C_3 European ecosystem (De Niro and Epstein, 1978; Schwarcz and Schoeninger, 1991), which is consistent with the Sado Valley environment. Their $\delta^{15}N$ values, 6.1‰ and 5.0‰ respectively, define the trophic baseline of the local food chain for the human palaeodietary reconstruction.

It is important to note that the lack of more analysed fauna could skew this reconstruction. In order to properly calculate the end-points for the interpretation of human dietary values, it was necessary to compare the data with faunal isotopic values from other contemporary Portuguese studies. Unfortunately, there are no useful isotopic data published on fauna from Portuguese Mesolithic sites, and these data are scarce in many other Mesolithic sites on the Atlantic coast near the Sado Valley sites, which could share a similar environment. In this context, the inclusion of the published isotopic data from more distant Atlantic sites (Table S1) allows the Portuguese faunal samples to be increased. The sites used for comparison are: the Mesolithic coastal site of La Vergne (Charente-Maritime, France) (Schulting, 2008); and the Mesolithic inland sites Noyen-sur-Seine (NW France) with terrestrial and riverine fauna (Bocherens et al., 2007; Drucker and Bocherens, 2004), and Pont d'Ambon (SW France) with some riverine fauna (Drucker and Bocherens, 2004).

A plot with four grey boxes with different faunal ecosystems was created with the data from Sado Valley human and faunal individuals, and the published data from the mentioned Atlantic sites (Fig. 3). These data were adjusted +1‰ for $\delta^{13}C$ and +3–5‰ for $\delta^{15}N$ to account for the diet-to-consumer shift, creating isotopic ranges (rectangular dotted line boxes) in which humans would end up if consuming faunal resources from grey boxes categories. Consumer isotopic values falling outside the dotted line boxes indicate a mixture of two or more different food sources. Marine box shifts were approximated from roughly contemporary sites in Atlantic Europe (Richards and Hedges, 1999; Schulting and Richards, 2001). Unfortunately, a more detailed reconstruction requires more faunal isotopic values, especially of freshwater and marine fauna.

Combining the faunal data, a theoretical terrestrial endpoint of ca. −21.3‰ (ca. −22.3‰ adjusted 1‰) can be suggested from the average $\delta^{13}C$ values, while the theoretical marine endpoint for species from the Atlantic Ocean is −12 ± 1‰ (Francalacci, 1989; Jennings et al., 1997; Richards and Hedges, 1999). The theoretical endpoint for terrestrial mammal protein consumption was calculated from the average $\delta^{15}N$ value of ca. 5.6‰ and, adjusted 3–5‰ for the trophic level shift correction, set a range of 8.6–10.6‰ (9–11‰, after rounding) for a significant animal protein intake in the human diet.

The average $\delta^{13}C$ values of adult human groups from the studied Sado sites are −19.4 ± 0.2 (1σ)‰ for Cabeço das Amoreiras, −20.0‰ for the only individual at Arapouco and −19.7 ± 0.3 (1σ)‰ for

Table 2
Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values obtained from faunal samples from the Portuguese sites: Cabeço das Amoreiras and Arapouco, indicating collagen control indicators (yield, %C, %N, C:N) and sampled bone.

Site	Individual	$\delta^{13}C$	$\delta^{15}N$	Yield (%)	%C	%N	C:N	Sampled bone
Cabeço das Amoreiras	*Oryctolagus cuniculus 1*	–	–	–	–	–	–	Scapula
	Oryctolagus cuniculus 2	–	–	–	–	–	–	Tibia diaphysis
	Cervus elaphus 1	–	–	–	–	–	–	Humerus diaphysis
	Cervus elaphus 2	–	–	–	–	–	–	Long bone diaphysis
	Cervus elaphus 3	−18.6	5.5	1.1	34.9	12.6	2.8*	Long bone diaphysis
	Cervus elaphus 4	–	–	–	–	–	–	Humerus diaphysis
	Capreolus sp.	−20.4	4.4	0.9	38.9	14.1	2.8*	Long bone diaphysis
	Bos primigenius 1	–	–	–	–	–	–	Long bone diaphysis
	Bos primigenius 2	–	–	–	–	–	–	Talus
	Bos primigenius 3	−19.0	6.1	1.7	40.7	13.9	2.9	Femur diaphysis
Arapouco	*Sus* sp.	−21.8	5.0	1.2	40.3	11.2	3.6	Long bone diaphysis

*Values that fall outside the accepted range for good collagen preservation.

Table 3
Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values obtained from human samples from the Portuguese sites: Cabeço das Amoreiras, Arapouco, Cabeço do Pez and Várzea da Mó, indicating sex and age, collagen control indicators (yield, %C, %N, C:N) and sampled bone.

Site	Individual	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Sex	Age	Yield (%)	%C	%N	C:N	Sampled bone
Cabeço das Amoreiras	*Skeleton 3*	−19.3	9.3	Male	Adult	2.2	44.0	15.1	2.9	Long bone diaphysis
	Skeleton 5	−19.0	9.5	Male	Adult	1.7	42.8	14.8	2.9	Long bone diaphysis
	Skeleton 6	−20.5	13.6	Male	Adult	0.6	46.3	11.8	3.9*	Humerus diaphysis
	Skeleton 7	−18.9	9.7	Male	Adult	1.5	37.0	12.8	2.9	Long bone diaphysis
Arapouco	*Skeleton 1*	–	–	Unknown	Adult	–	–	–	–	Rib
	Skeleton 3A	–	–	Unknown	Adult	–	–	–	–	Humerus diaphysis
	Skeleton 5	−20.0	9.4	Female?	Adult	1.1	39.9	13.1	3.0	Tibia diaphysis
	Skeleton 7	–	–	Unknown	Adult	–	–	–	–	Long bone diaphysis
Cabeço do Pez	*Skeleton 2*	−18.4	11.9	Unknown	1.5–2 y.	1.2	35.9	11.1	3.2	Fibula diaphysis
	Skeleton 3A	–	–	Unknown	Adult	–	–	–	–	Fibula diaphysis
	Skeleton 9	−20.0	9.0	Unknown	Adult	1.4	43.5	14.9	2.9	Rib
	Skeleton 10	–	–	Unknown	Adult	–	–	–	–	Tibia diaphysis
	Skeleton 17	−19.4	9.3	Female	5–7 y.	1.9	43.6	14.9	2.9	Long bone diaphysis
	Skeleton 21	−19.3	9.2	Female	Adult	2.1	35.2	12.1	2.9	Humerus diaphysis
	Skeleton 27	−19.5	10.0	Male	Adult	2.4	42.0	14.4	2.9	Humerus diaphysis
	Skeleton A	−20.0	9.3	Male	Adult	2.3	39.1	13.3	2.9	Cranium
	Skeleton B	−19.8	8.2	Male	Adult	1.5	38.7	13.5	2.9	Cranium
Várzea da Mó	*Skeleton ?*	–	–	Unknown	Adult	–	–	–	–	Long bone diaphysis

*Values that fall outside the accepted range for good collagen preservation.

Cabeço do Pez. These values are consistent with a diet based mainly on C_3 terrestrial resources. The average $\delta^{15}N$ values are $9.5 \pm 0.2(1\sigma)$‰, 9.4‰ and $9.1 \pm 0.6(1\sigma)$‰ for each site respectively, placing humans on a clearly higher trophic level than the herbivores (almost 4‰ higher).

Setting the terrestrial and marine endpoints at −21‰ to −12‰ and taking into account the human $\delta^{13}C$ values obtained here, a low proportion of marine proteins (approximately 10–20%) were consumed by Sado Valley individuals. Considering the obtained $\delta^{15}N$ values from Sado Valley humans, there is no trace of freshwater protein consumption in their diet.

The study of the two sub-adults from Cabeço do Pez (Table 3 and Fig. 2) shows that the isotopic signature of Skeleton 2, a 1.5–2 year-old individual of unknown sex, displays a higher $\delta^{15}N$ value, while the $\delta^{13}C$ value is similar to the rest of the population. This is in contrast with Skeleton 17, a 5–7 year-old female, who shows the same diet as the adults in her group. The nitrogen signature of Skeleton 2 has a specific increase of 3‰, which may indicate that this individual was still in the nursing period when he or she died (Katzenberg et al., 1996; Balasse et al., 2001). However, the nitrogen signature of Skeleton 17, similar to the adult population, probably suggests that this individual was weaned years before her death, and started to consume the same food as adult individuals.

Finally, there are no significant isotopic differences between males and females at Cabeço do Pez, the only site where both sexes have been analysed. The probable female from Arapouco is the only

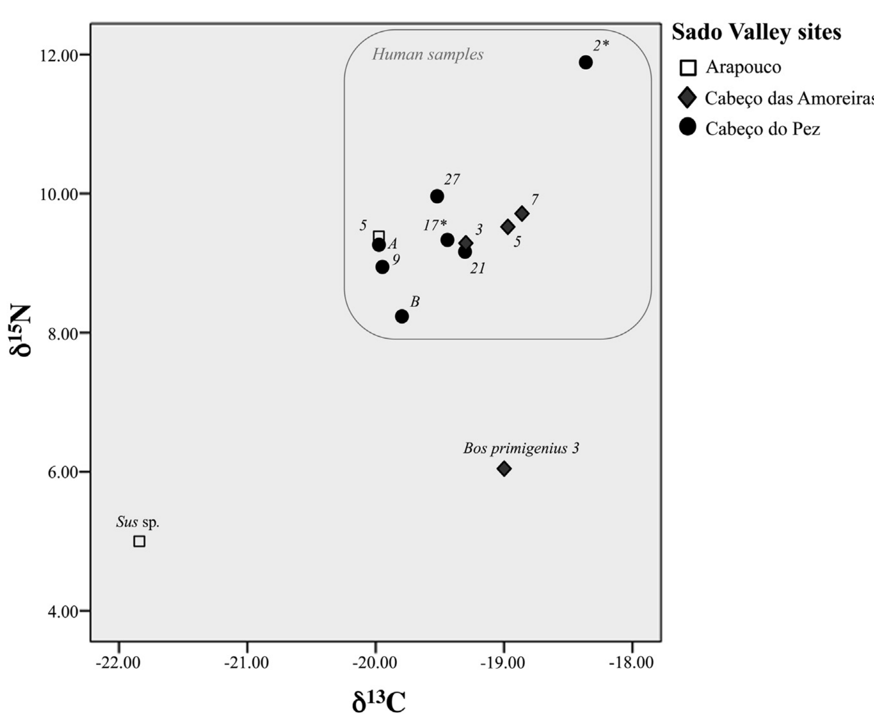

Fig. 2. Plot of $\delta^{13}C$ and $\delta^{15}N$ values, expressed in ‰, of bone collagen from human adults, sub-adults (*) and fauna from the Portuguese Mesolithic sites: Cabeço das Amoreiras, Arapouco and Cabeço Pez.

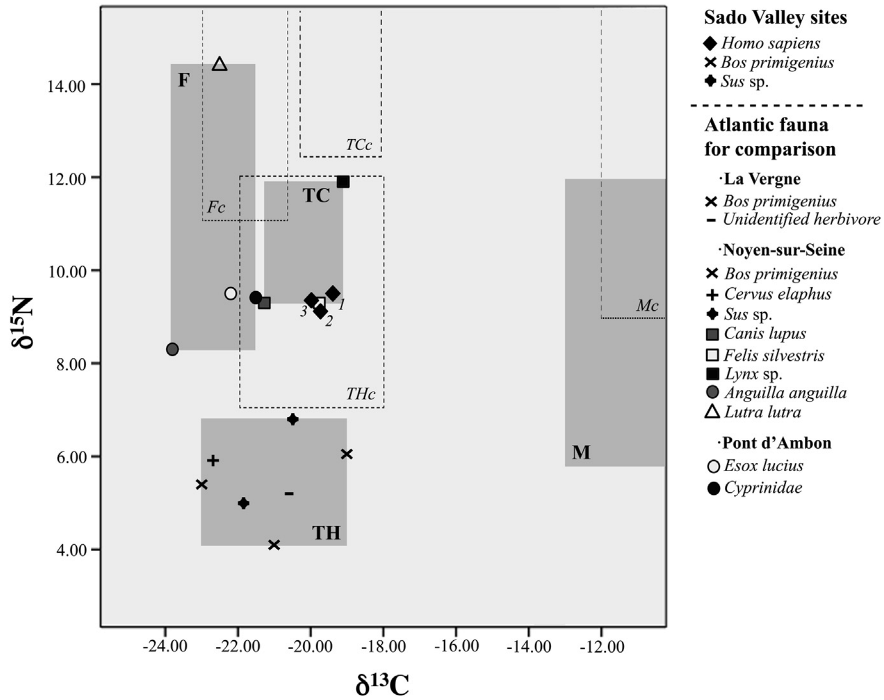

Fig. 3. Plot of δ^{13}C and δ^{15}N average values, expressed in ‰, of Sado Valley sites (1. Cabeço das Amoreiras; 2. Cabeço do Pez; and 3. Arapouco) and the values of their fauna, with the stable isotope data of other Atlantic fauna used for comparison. Data points are grouped according to their habitat and position in the food web (rectangular grey boxes). The rectangular dotted line boxes are taken from the faunal rectangular grey boxes and have been shifted by +1‰ on δ^{13}C axis and +3–5‰ on δ^{15}N axis to account for the trophic shift in isotope values from food source to consumer. F, TH, TC and M = Freshwater fauna, Terrestrial Herbivores, Terrestrial Carnivores and Marine fauna, respectively. Fc, THc, TCc and Mc = Freshwater resources consumers, Terrestrial Herbivores consumers, Terrestrial Carnivores consumers and Marine resources consumers, respectively.

individual at that site, and therefore does not provide enough information on this matter.

6. Discussion

6.1. The individuals from the Sado Valley: Cabeço das Amoreiras, Arapouco and Cabeço do Pez

The isotopic data from the three Sado Valley sites suggest a relatively homogeneous terrestrial C_3 diet mainly dominated by the consumption of meat proteins from terrestrial herbivores and, to a lesser extent, from vegetable protein from C_3 terrestrial-based resources. There is a little but clear isotopic evidence of marine protein intake of about 10–20% at all three sites, but there is no clear contribution of freshwater resources at any of the sites.

The contribution of terrestrial resources, from both animals and vegetables, and the limited marine protein intake in the diet of Sado Valley individuals is corroborated by a previous isotopic study of Skeleton 3 (δ^{13}C −18.5‰ and δ^{15}N 9.49‰) from Cabeço das Amoreiras and Skeleton 27 (δ^{13}C −18.7‰ and δ^{15}N 9.83‰) from Cabeço do Pez (Cunha and Umbelino, 2001) and the trace element analyses performed on 51 individuals from Sado Valley (Umbelino, 2006; Umbelino et al., 2007). The marine resources intake in Sado individuals is also supported by archaeological data, which describes the recovery of a large amount of fish and crustacean remains, besides the shellfish of the shell middens, especially in Arapouco (Arnaud, 1987, 1989).

The species of terrestrial mammals that significantly contribute to the Mesolithic human diet in these regions are *B. primigenius*, *Cervus elaphus* and *Sus scrofa*; and, as occasional sources, *Oryctolagus cuniculus* and *Lepus capensis*, according to the little amount of biomass found of these small mammals (Rowley-Conwy, 1983; Cunha, 2002–2003; Umbelino and Cunha, 2012). According to palynological studies, between 6595-6430 cal BC and 5628–5376 cal BC, the environment was characterized by the presence of thick forests of *Pinus pinaster*, *Quercus faginea*, *Olea europaea sylvestris* and *Ceratonia siliqua*, which were gradually reduced and replaced by shrubby vegetation by 5628–5376 cal BC (Mateus, 1985; Araújo, 1999; Umbelino and Cunha, 2012). The nutritional contribution of vegetable protein could come from the abundant plant sources present in the environment such as tubers, legumes and fruits (e.g. acorns, carobs and berries), but more analyses are required to determine which plants contributed to the diet of the humans from Sado Valley. The marine species most likely consumed by the studied individuals are: shellfish, with 90% of the marine faunal spectrum consisting of Verenoids, e.g. *Cerastoderma edule* found at the three sites but to a greater extent in Cabeço das Amoreiras (Albizuri, 2010); and low trophic level marine fish (e.g. *Sparus aurata* and *Argyrosomus regius*) according to remains found in Arapouco.

There is great similarity in the isotopic ratio among the individuals who were buried at the same site, and slight dietary differences are observed among the three groups. These differences, corroborated by the above mentioned trace element analyses (Umbelino, 2006; Umbelino et al., 2007), could be explained by each site belonging to a single population group where the three sites behaved as distinct communities.

The palaeodietary reconstruction of Sado Valley groups has also led to hypotheses about their possible subsistence economy and social aspects. In this context, the possible mobility of each group between their hypothetical inland settlements to different periodical settlements in the estuarine landscape seems feasible. According to the small contribution of marine protein consumption, the different communities would have moved to the estuary in favourable seasons in order to diversify their diet by exploiting the marine resources available. These communities were well adapted

Fig. 4. Location of the Mesolithic sites used for the study.

to the Sado microenvironment where they could efficiently procure all the available terrestrial and marine resources.

The favourable seasons for fishing and gathering marine resources were probably spring and summer, and in some cases autumn, when waters were warmer. In these seasons low trophic level marine fish enter the Portuguese estuaries to feed and breed, and bivalves start reproducing, which provides a stable source of marine resources in the estuary. Therefore, the increasing availability of estuarine resources and the stability of the valley floor environments allowed semi-sedentary occupations in these regions (Van der Shriek et al., 2007).

The relative homogeneity of the size of shells suggests a temporary and controlled exploitation of the river delta (Albizuri, 2010). The shells found in Sado Valley exhibit evidence of intensive foraging of molluscs with their habitat in sandy and tidal areas. The selective foraging of *C. edule* specimens, with a similar average of 19.29 mm in height and 21.21 mm in width (Dean, 2010), suggests that it may have occurred at the same time of the year. Subsequent studies of malacofauna and the environmental conditions of the Sado sites would provide more information about the time of harvesting these bivalves. Nevertheless, modern specimens of *C. edule* can reach about 45 mm on Portuguese beaches (Dean, 2010) and the archaeological samples are on the small side of their size range, which suggest several hypotheses: an early harvesting of these resources; a *C. edule* size reduction prompted by the Mesolithic environment, which could result in lower human foraging efficiency, or an over-exploitation of these resources. Another hypothesis for the small size of *C. edule* is that it could be due to a strategy to avoid poisoning caused by Harmful Algal Blooms (HAB), commonly called “red tides”. The “red tide” could occur in low-tide conditions during the summer, when salinity increases due to high temperatures and reduced rainfall. These conditions lead to a

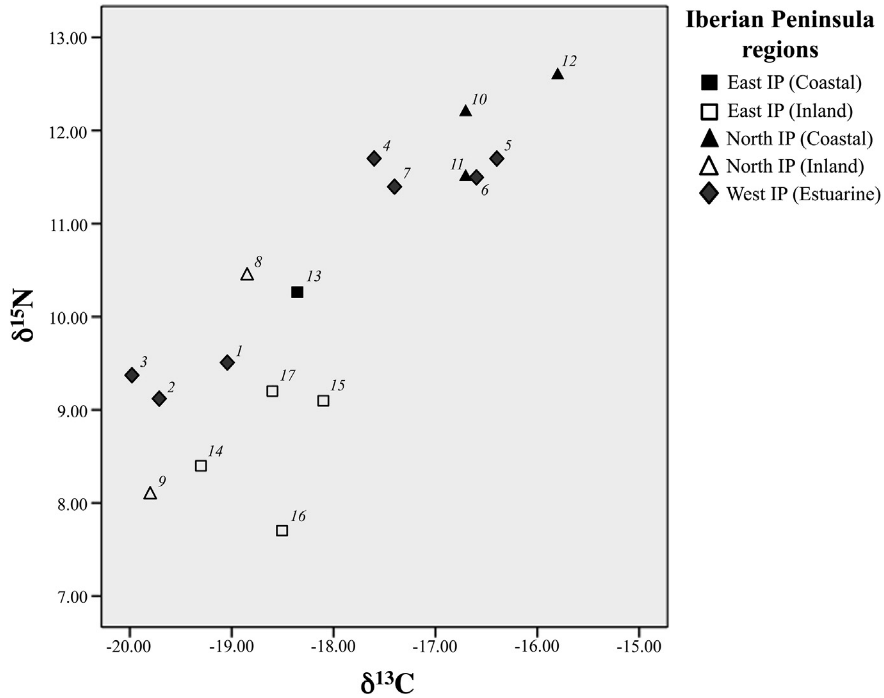

Fig. 5. Plot of $\delta^{13}C$ and $\delta^{15}N$ values, expressed in ‰, of the Mesolithic remains from five sites of the Cantabrian region (northern Iberian Peninsula), five sites of the Mediterranean region (eastern Iberian Peninsula) and seven sites of the Atlantic region (Muge and Sado Valley, Portugal): Cabeço das Amoreiras (1), Cabeço do Pez (2), Arapouco (3), Poças de São Bento (4), Moita do Sebastião (5), Cabeço da Amoreira (6), Cabeço da Arruda (7), La Braña-Arintero (8), Los Canes (9), Poza L'Egua (10), J3 (11), Colomba (12), El Collado (13), Casa Corona (14), Santa Maira (15), Penya del Comptador (16) and Cingle del Mas Nou (17).

massive multiplication of the dinoflagellate *Dinophysis*, which causes Diarrhetic Shellfish Poisoning (DSP). If this occurred in Portugal in the Mesolithic (Vale and Sampayo, 2003; Jackes and Meiklejohn, 2008), individuals in the Sado Valley, well adapted to the microenvironment of the estuary, may have been aware of the toxic effects of the poisoned shellfish and restricted their collection to periods before the appearance of this episode. Strategies related to the “red tide” have been studied in prehistoric individuals from Western Cape in Africa (Parkington et al., 2009). Nevertheless, this hypothesis should be considered with caution, as further palaeoecological data from this area is required.

The collection of a wide variety of shellfish in Early Mesolithic sites was replaced by intensive harvesting of certain species in the Late Mesolithic (Dean et al., 2011). This concurs with the chronology and characteristics of the studied sites, considering the abovementioned selective foraging of Verenoids. Nevertheless, these economic shifts were apparently experienced as significant resource depressions by Mesolithic shell fishers, rather than a decrease in the foraging efficiency in the use of marine resources, characteristic of the end of the Late Mesolithic and the onset of the Neolithic period (Dean et al., 2011).

It is worth noting that the hypotheses about these periodical settlements, although reinforced by some archaeological and bioanthropological studies (Arnaud, 1987, 1989; Umbelino, 2006; Umbelino et al., 2007; Umbelino and Cunha, 2012), require additional radiocarbon dating in human remains and an extensive study of the archaeological context and the burial practices at each site.

6.2. *Comparison of the Portuguese sites with the Iberian Peninsula and with other Mesolithic groups from the rest of Europe*

The comparison of the Portuguese samples analysed here with 14 Mesolithic sites from the Iberian Peninsula and 25 Mesolithic sites from the rest of Europe (all of them indicated in Fig. 4 and listed in Table S2), allows defining dietary and economic patterns in the European Mesolithic period within the thresholds established for the studied geographical areas.

For the purpose of such comparisons, the different types of sites are defined as follows. A site is considered estuarine when it is located far from the coast, within the tidal reach of the river, at a lower or higher altitude depending on the site. The coastal sites are defined in the present study as the sites located less than 10 km from the coast. The landscape variations that have occurred since the Mesolithic may have increased this distance. Finally, inland sites are defined as areas where access to the coast might be difficult, because of the geomorphology of the area or the distance (more than 10 km away, although in some cases the local geomorphology means this threshold has to be reduced, because contact with the coast is unfeasible).

6.2.1. *Comparison of the Portuguese sites within the Iberian Peninsula*

The Iberian sites were grouped according to their region: Eastern inland and coastal sites, Northern inland and coastal sites and Western estuarine sites (Fig. 5).

There is no clear uniformity in the diet at the different Mesolithic sites in the Iberian Peninsula. However, to a greater or lesser extent, it seems that a direct relationship exists between the proximity of the coast or estuary and the consumption of marine resources in the diet at some Mesolithic Iberian sites. In some inland sites, however, this relationship is not so straightforward. The Mediterranean site Santa Maira (15) and some individuals from Cingle del Mas Nou (17), show a diet based primarily on terrestrial resources, but with a slight contribution of marine proteins. This low proportion of marine proteins may indicate mobility of these individuals between sites located in mid-mountain areas and the coast (Salazar-García et al., 2014). The two individuals at the inland site of La Braña-Arintero (8) exhibit $\delta^{13}C$ values equivalent to the

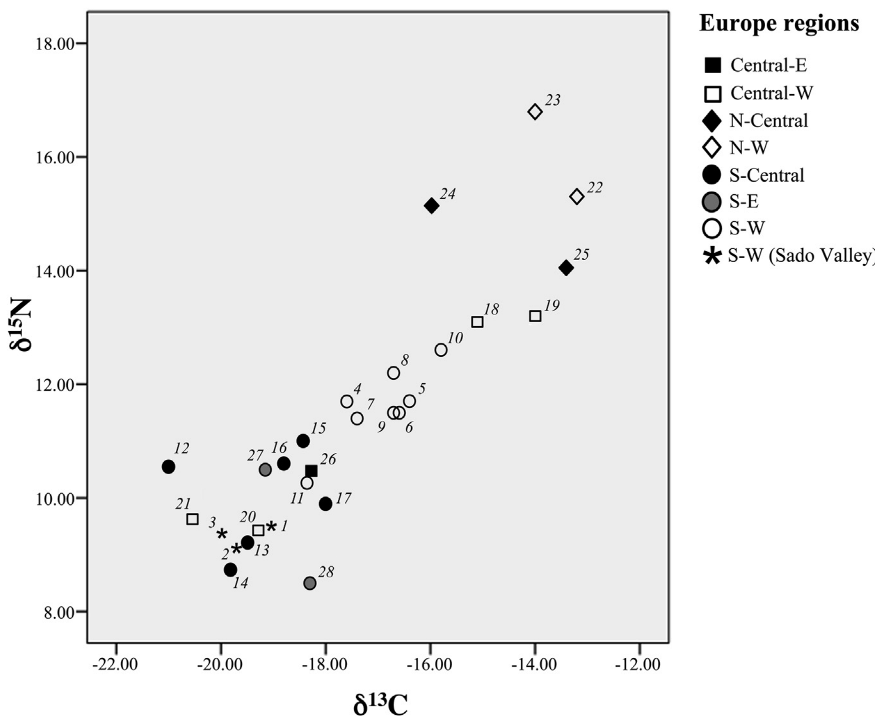

Fig. 6. Plot of $\delta^{13}C$ and $\delta^{15}N$ values, expressed in ‰, of the Mesolithic remains from European sites located near the coast or esturaries: Cabeço das Amoreiras (1), Cabeço do Pez (2), Arapouco (3), Poças de São Bento (4), Moita do Sebastião (5), Cabeço da Amoreira (6), Cabeço da Arruda (7), Poza L'Egua (8), J3 (9), Colomba (10), El Collado (11), Grotta dell'Uzzo (12), Grotta Addaura Caprara (13), Grotta della Molara (14), Grotta d'Oriente (15), Araguina Sennola (16), Monte Leone (17), Téviec (18), Hoëdic (19) La Vergne (20), Meuse Basin (21), Oronsay sites (22), Ferriter's Cove (23), Swedish coastal sites (24), Danish coastal sites (25), Fat'ma koba (26), Pupi'cina (27) and Vela Spilja-Vela Luka (28).

consumption of marine protein. In this case, the authors suggest that these individuals most probably consumed a largely terrestrial diet, and the relatively high carbon values were the consequence of other factors (Arias and Schulting, 2010).

A larger consumption of marine resources in the diet at Muge and Cantabrian coastal sites in comparison with sites located further south in the centre of the Iberian Peninsula can also be appreciated. The Cantabrian coastal sites show a balanced diet of approximately 50-50% marine and terrestrial proteins (Arias, 2005). In the Atlantic region, the Portuguese estuarine sites in Muge and the other published site in the Sado Valley (Poças de São Bento), show a higher consumption of seafood (>25%) than the individuals from the Mediterranean coast (Lubell et al., 1994; Umbelino, 2006; Roksandik, 2006). The large contribution of marine resources observed in these Portuguese estuarine sites contrasts with the low proportion of marine protein intake in the studied Sado Valley sites.

Individuals from Muge and Sado would be expected to have a similar diet, since they are both located in estuaries, of practically the same chronology, and fairly close to each other in comparison with other populations in the Iberian Peninsula. In this context, the general trend of an increased consumption of marine resources in the advanced stages of the Mesolithic (Bonsall et al., 1997; Schulting and Richards, 2002; Richards et al., 2005; Fischer et al., 2007) does not agree with what has been found at the sites studied here.

In this sense, the differences observed in the diet of the groups from Sado Valley could be due to diversified subsistence strategies fostered by demographic and social factors. As discussed above, the location of hypothetical inland settlements is unknown, but probably some kilometres from the coast and the riverbank. For this reason, their forays to the estuarine landscape were probably limited, and they presumably went there only occasionally, in order to increase the variety in their diet.

6.2.2. Comparison between the Portuguese sites and other Mesolithic populations of Europe

A further comparative study was made of our new data, sites on the Iberian Peninsula, and sites from elsewhere in Europe (Table S2), which represents a wide range of subsistence models in this period.

The $\delta^{13}C$ and $\delta^{15}N$ values at different Mesolithic coastal and estuarine sites in Europe, in a similar location to the Sado Valley sites, are plotted in Fig. 6.

A heterogeneous diet can be noticed among the different coastal and estuarine European sites, although an interesting gradient is observed in the animal protein intake from Southern to Northern sites. Meat consumption increases as the sites are located further north as well as in areas where the consumption of marine mammals might be widespread (e.g. the Brittany sites 18 and 19, Fig. 6). In these cases, the successful use of the available seafood leads to a human protein diet consisting almost entirely of marine resources.

In these Northern and colder regions, the consumption of animal proteins and lipids became essential to the livelihood of its residents. The continual consumption of animal meat and fat would provide useful energy for acclimation to the cold and, consequently, better adaptation of those individuals with greater tolerance to this kind of diet. It is also important to highlight the scarcity of plant foods during the coldest periods, when snow prevented plants from growing. In this context, the subsistence pattern of Northern societies should be discussed considering both scenarios: an increased consumption of terrestrial animal proteins due to more hunting; or, as can be observed in the isotopic values of the compared sites, a higher consumption of marine resources. This higher marine protein intake came from fishing and gathering marine resources, or from hunting marine mammals such as seals.

At lower latitudes, a dietary downward gradient of $\delta^{13}C$ values and in the contribution of animal resources can be observed. This

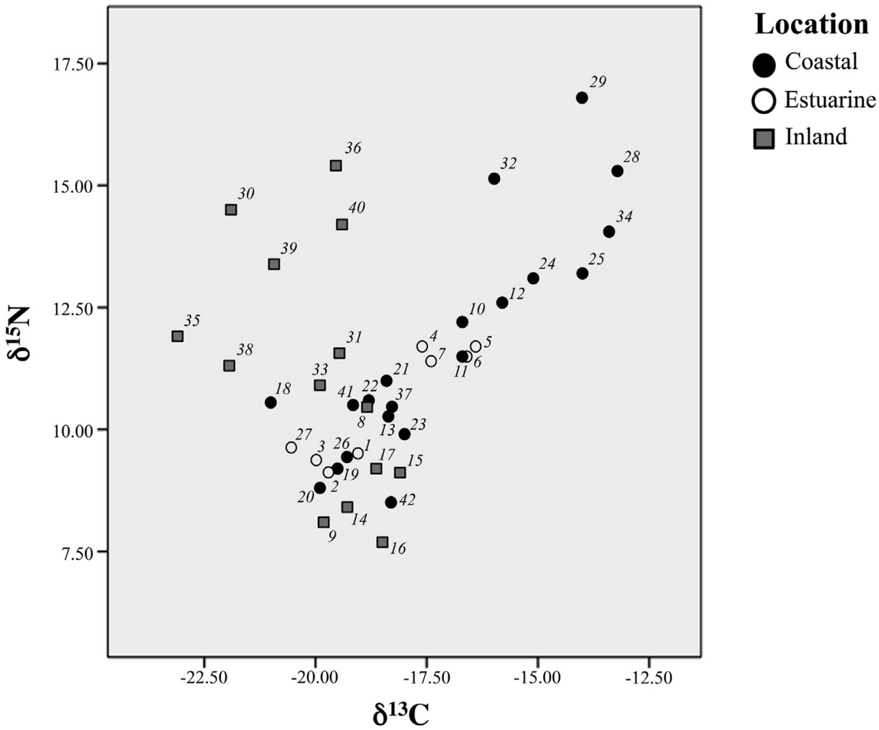

Fig. 7. Plot of $\delta^{13}C$ and $\delta^{15}N$ values, expressed in ‰, of the Mesolithic remains from European sites: Cabeço das Amoreiras (1), Cabeço do Pez (2), Arapouco (3), Poças de São Bento (4), Moita do Sebastião (5), Cabeço da Amoreira (6), Cabeço da Arruda (7), La Braña (8), Los Canes (9), Poza L'Egua (10), J3 (11), Colomba (12), El Collado (13), Casa Corona (14), Santa Maira (15), Penya del Comptador (16), Cingle del Mas Nou (17), Grotta dell'Uzzo (18), Grotta Addaura Caprara (19), Grotta della Molara (20), Grotta d'Oriente (21), Araguina Sennola (22), Monte Leone (23), Téviec (24), Hoëdic (25), La Vergne (26), Meuse Basin (27), Oronsay sites (28), Ferriter's Cove (29), Hardinxveld (30), Swedish inland sites (31), Swedish coastal sites (32), Danish inland sites (33), Danish coastal sites (34), Zvejnieki (35), Schela Cladovei (36), Fat'ma koba (37), Marievka (38), Vasilievka II (39), Vlasac (40), Pupi'cina (41) and Vela Spilja-Vela Luka (42).

gradient ranges from individuals with a significant animal protein intake to those with an occasional intake of this type of food. In these cases, the marine diet of the individuals is lower, but evident, being balanced with terrestrial prey or regional plant foods.

In sites at increasingly lower latitudes, the animal protein component of the diet is considerably reduced and vegetable intake is more important, in agreement with the richness and variety of plants present at these latitudes, like the Sicily sites, Grotta Addaura Caprara (13) and Grotta della Molara (14). Nevertheless, to a greater or lesser degree, some of these communities include a slight contribution of marine foods that could have enriched the diet of the consumers. But, in some other cases, this proportion of marine resources came from a smaller amount of marine foods that could not be detected isotopically, e.g. the individuals from Vela Spilja-Vela Luka (28) (Lightfoot et al., 2011).

Finally, all the Mesolithic sites in Europe, including all the locations (coastal, estuarine and inland) are plotted in Fig. 7. This comparison provides a more complete view of the subsistence patterns of the studied individuals according to their geographical location.

There is a clear separation between groups living in inland regions and those living on the coast or near estuaries, except for the Iberian cases 8, 9, 14, 15, 16 and 17 (Fig. 7): La Braña-Arintero, with the aforementioned unusual human isotopic signatures for an inland site, Los Canes, Casa Corona, Santa Maira, Penya del Comptador and Cingle del Mas Nou, respectively. These sites, with the exception of La Braña Arintero, have low nitrogen levels compared to the other studied sites and display a diet based mainly on the intake of vegetable proteins. The consumption of meat was relegated to the occasional hunting of large terrestrial herbivores. The slight contribution of marine proteins for Santa Maira and some individuals from Cingle del Mas Nou plotted these two sites together with coastal sites with a slight increase in carbon values.

Generally, the diet of the inland sites is clearly different from those discussed above, maintaining low values of carbon isotope and variable values of nitrogen. These signatures indicate terrestrial diets that have an important contribution of animal proteins, but also a significant intake of plant resources. The animal contribution comes mainly from the hunting of terrestrial mammals and can increase in societies with greater availability of such resources. Nevertheless, in some cases with lower carbon signatures, the high levels of nitrogen could mask animal protein intake from freshwater sources, such as invertebrates and fish from rivers or lakes. In cases 30 and 39 (Fig. 7), it appears that animal proteins and freshwater fish were important dietary elements, while in cases 35, 36, 38 and 40 (Fig. 7), the diet appears to have been mixed to a greater or lesser extent, which is probably associated with a variable habitation regime over the course of the year.

The complex diets reveal the difficulty of palaeodietary studies. As can be seen in the Sado Valley sites, the duration of settlement depends on the available resources, which depend on the season and the geographical area. This highlights the importance of environmental and biological studies of each area during the analysed period. Social and demographic factors should always be considered, especially in those cases where the human palaeodietary signatures are not fully in agreement with the use of local food sources or the environment where the individuals lived.

7. Conclusions

The stable isotope analyses of the three Mesolithic human communities from Sado Valley revealed that they consumed a

diverse diet mainly consisting of terrestrial C_3 resources, both animal and vegetable, and a small proportion of marine resources close to 20%. The communities seem to be well adapted to the Sado microenvironment, where they could efficiently procure all the available terrestrial and marine resources out of the wide spectrum of the available food that characterized this region in the Mesolithic. In the context of palaeodietary reconstruction, human mobility between hypothetical inland settlements and other temporary settlements near the Sado estuary seems feasible.

The regional dietary heterogeneity seen in all the communities that are discussed in this paper hampers the characterization of a single subsistence pattern for all Mesolithic groups. But it seems clear that the Mesolithic communities in western Iberia were much less dependent on marine foods than contemporary populations in other areas of the Iberian Peninsula and northern Europe. According to the data, the subsistence strategy of the Mesolithic period could be characterized by a high degree of regionalization. Several factors produce significant variations in the subsistence of Mesolithic communities. These factors include: the availability of the resources and the environmental characteristics of each site, to which individuals should be adapted; the more or less effective management techniques for procuring resources; and the important influence of demographic and social factors which could lead to dietary preferences and, therefore, to inconsistencies between the edible resources and individuals' diet.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2014.07.028.

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